

process shall be repeated for the injection of the third group of embryos using the yolk sacs of viable embryos from the second group.

(3) For each of the three passages, embryo deaths occurring within 48 hours of injection shall be disregarded, except that if more than three such deaths occur at any passage, that passage shall be repeated.

(b) If one or more embryo deaths occur at any passage after 48 hours postinjection, the yolk sacs from each of the dead embryos shall be subcultured into 10 additional embryos. If one or more embryo deaths again occur due to chlamydial agents, the Master Seed Virus is unsatisfactory for use to produce vaccine.

[44 FR 58899, Oct. 12, 1979]

§ 113.44 Swine safety test.

The swine safety test provided in this section shall be conducted when prescribed in a Standard Requirement or in the filed Outline of Production for a product.

(a) *Test procedure.* (1) Inject each of two swine of the minimum age for which the product is recommended with the equivalent of two doses of bacterial vaccine or 10 doses of viral vaccine.

(2) Administer vaccine in the manner recommended on the label.

(3) Observe swine each day for 21 days.

(b) *Interpretation.* If unfavorable reactions attributable to the product occur in either of the swine during the observation period, the serial or subserial is unsatisfactory. If unfavorable reactions which are not attributable to the product occur, the test shall be declared inconclusive and may be repeated; *Provided*, That, if the test is not repeated, the serial or subserial shall be declared unsatisfactory.

[48 FR 33476, July 22, 1983]

§ 113.45 Sheep safety test.

The sheep safety test provided in this section shall be conducted when prescribed in a Standard Requirement or in the filed Outline of Production for a product.

(a) *Test procedure.* (1) Inject each of two sheep of the minimum age for

which the product is recommended with the equivalent of two doses of bacterial vaccine or 10 doses of viral vaccine.

(2) Administer vaccine in the manner recommended on the label.

(3) Observe sheep each day for 21 days.

(b) *Interpretation.* If unfavorable reactions attributable to the product occur in either of the sheep during the observation period, the serial or subserial is unsatisfactory. If unfavorable reactions which are not attributable to the product occur, the test shall be declared inconclusive and may be repeated; *Provided*, That, if the test is not repeated, the serial or subserial shall be declared unsatisfactory.

[48 FR 33476, July 22, 1983]

§ 113.46 Detection of cytopathogenic and/or hemadsorbing agents.

The tests for detection of cytopathogenic and/or hemadsorbing agents provided in this section shall be conducted when prescribed in an applicable Standard Requirement or in the filed Outline of Production for a product.

(a) *Test for cytopathogenic agents.* One or more monolayers that are at least 6 cm² and at least 7 days from the last subculture shall be tested as provided in this paragraph.

(1) Stain each monolayer with a suitable cytological stain.

(2) Examine the entire area of each stained monolayer for evidence of inclusion bodies, abnormal number of giant cells, or other cytopathology indicative of cell abnormalities attributable to an extraneous agent.

(b) *Test for hemadsorbing agents.* One or more monolayers that are at least 6 cm² and at least 7 days from the last subculture shall be tested as provided in this paragraph.

(1) Wash the monolayer with several changes of phosphate buffered saline.

(2) Add an appropriate volume of a 0.2 percent red blood cell suspension to uniformly cover the surface of the monolayer of cultured cells. Suspensions of washed guinea pig and chicken red blood cells shall be used. These suspensions may be mixed before addition to the monolayer or they may be added separately to individual monolayers.